# SCANNING ELECTRON MICROSCOPY AS AN AID TO POLLEN TAXONOMY[1,2]

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ABSTRACT

Information obtained from scanning electron microscopy of whole pollen grains of *Zea* and *Iva* indicates direct correlation with that gathered from light microscopy and electron microscopy of thin sections. This information helps clarify and support past analyses.

## INTRODUCTION

The portrayal of pollen surfaces has been greatly improved by the scanning electron microscope. Although still a relatively new instrument, there are already several publications describing its utility in pollen and spore investigations (see Echlin, 1968, for an extensive bibliography).

For a number of years one of us (J.J.S.) has been concerned primarily with the morphology and taxonomy of grass and composite pollen. This has involved studies with the light microscope and the transmission electron microscope. Since the scanning electron microscope offers a rapid means of observing pollen wall surfaces, as well as providing greater resolution than the light microscope, the importance of this instrument to our work is obvious. Although preparative techniques for the scanning electron microscope are relatively simple and have been described (Echlin, 1968), comparatively little information is available on the two major processing techniques employed in taxonomic studies of pollen (viz. fresh and acetolyzed). Moreover, the aperture regions, so critical in taxonomic diagnoses, have been neglected at the expense of the unaperturate portions of the pollen wall. This report illustrates the effectiveness of scanning electron microscopy in studies of pollen morphology.

## MATERIALS AND METHODS

Fresh pollen of *Zea mays (Gramineae)* and species of *Iva (Compositae)* were used. The pollen was examined in three stages: (1) fresh, unstained, (2) fresh, stained with $OsO_4$, and (3) acetolyzed. After $OsO_4$ staining or acetolysis, the pollen was dehydrated through graded alcohols and allowed to dry after final rinses in either propylene oxide or reagent grade acetone.

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Preparation for the scanning electron microscope consisted of dusting the dried pollen onto specimen stubs which held a piece of double-stick Scotch tape. The stubs were then placed in a Kinney vacuum evaporator and shadowed with chromium. During shadowing the stubs were continually rotated through 360 degrees. After 30 - 45 seconds of evaporation the pollen was ready for observation in the scanning electron microscope. A Stereoscan Mk IIa Scanning Reflection Electron Microscope was used to analyze the pollen.

## RESULTS

*Zea mays.* Fresh, untreated pollen showed a high percentage of wrinkled, collapsed and exploded grains. However, details of the pore region even in distorted grains proved to be instructive. In Figure 1 an essentially intact pore clearly shows the raised annulus surrounding a depressed operculum. The latter is ornamented with spinules which are similar to those present on the pollen surface. In Figure 2 the operculum is missing, probably due to the high vacuum environment during shadowing. The angle of tilt of this grain is such that a differentiation in electron conductance is evident along one portion of the pore circumference. This difference in pollen wall density is believed to reflect the bilayered nature of the pore. Such an interpretation is consistent with transmission electron microscope studies of thin sections (Skvarla and Larson, 1966).

When fresh pollen was treated with $OsO_4$ for approximately 45 minutes the percentage of distorted grains was appreciably decreased. The preservation of the pore region, however, was not significantly improved and was comparable to fresh untreated grains. For example, the endexine layer which normally traverses the gap beneath the operculum and consequently is not visible in surface view, can, by virtue of operculum tearing, be clearly distinguished (Fig. 3).

Acetolysis demonstrated a stability comparable to that achieved with $OsO_4$ treatment. Opercula were nearly always absent (due to acetolysis) but pore and exine details provided results identical to those by the above processing treatments (Fig. 4).

*Iva.* Composite pollen, because of its robust exine, is substantially more resistant than *Zea* to distortion during evaporation and in the electron beam. The greatest difficulty in working with this pollen was in analyzing grains with extensive colpi. In fresh, untreated *Iva xanthifolia* (Fig. 5) the colpus and pore were photographed almost immediately after initial observation. When a second exposure was attempted a few minutes later, the flanks of the colpus had become relaxed and closed (Fig. 6), thus obscuring the pore. This tendency of the colpus/pore region to fold inward was frequently noted in fresh untreated pollen (Fig. 7). With the application of a short $OsO_4$ treatment the colpus showed less of a tendency toward closing.

Beyond question, acetolyzed pollen provided the most clearly defined colpus-pore data. Grains were nearly always expanded and pore configurations and exine layering within the colpus were readily distinguishable (Figs. 8-9).

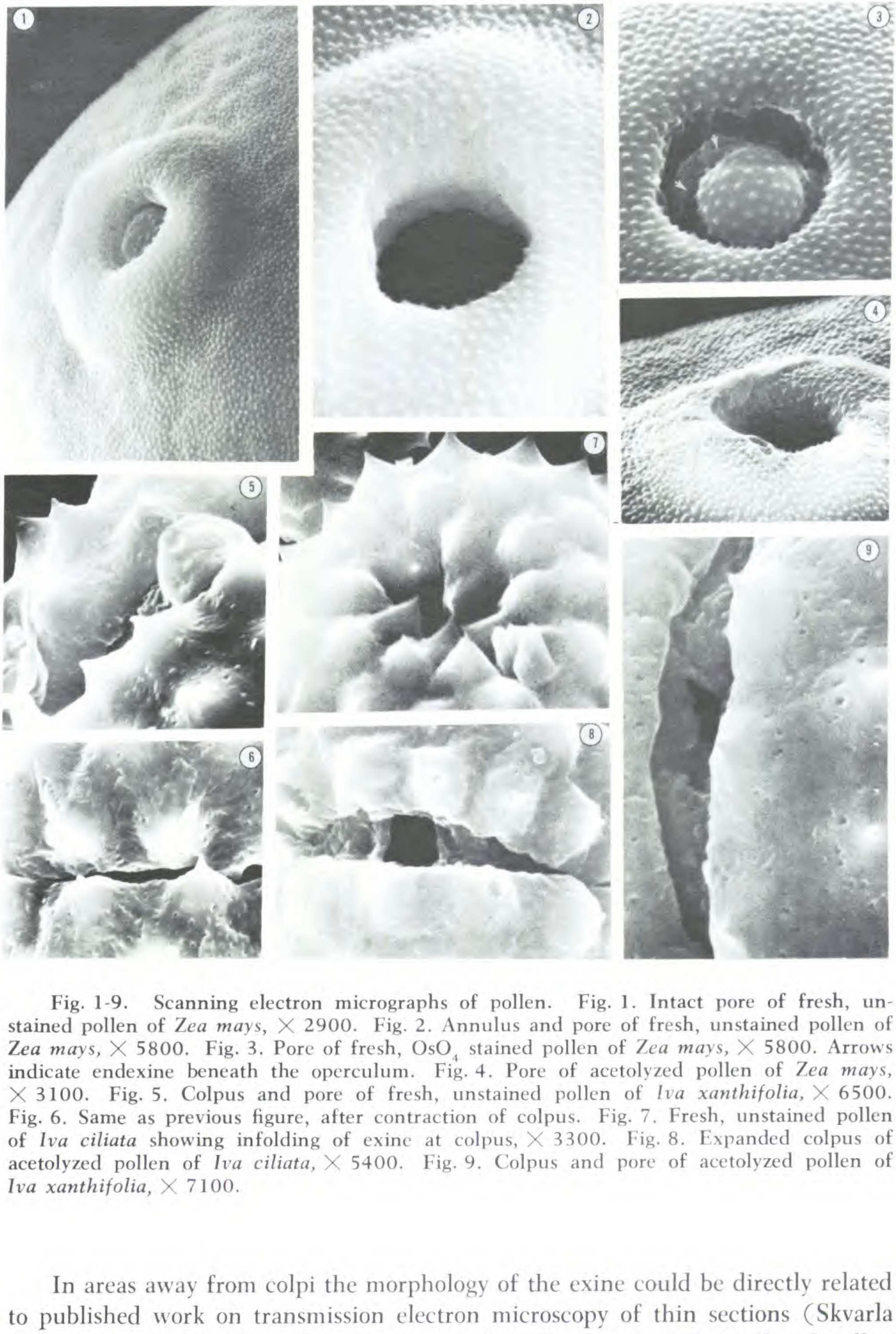


Fig. 1-9. Scanning electron micrographs of pollen. Fig. 1. Intact pore of fresh, unstained pollen of *Zea mays*, × 2900. Fig. 2. Annulus and pore of fresh, unstained pollen of *Zea mays*, × 5800. Fig. 3. Pore of fresh, $OsO_4$ stained pollen of *Zea mays*, × 5800. Arrows indicate endexine beneath the operculum. Fig. 4. Pore of acetolyzed pollen of *Zea mays*, × 3100. Fig. 5. Colpus and pore of fresh, unstained pollen of *Iva xanthifolia*, × 6500. Fig. 6. Same as previous figure, after contraction of colpus. Fig. 7. Fresh, unstained pollen of *Iva ciliata* showing infolding of exine at colpus, × 3300. Fig. 8. Expanded colpus of acetolyzed pollen of *Iva ciliata*, × 5400. Fig. 9. Colpus and pore of acetolyzed pollen of *Iva xanthifolia*, × 7100.

In areas away from colpi the morphology of the exine could be directly related to published work on transmission electron microscopy of thin sections (Skvarla and Larson, 1966). In such areas untreated, $OsO_4$-treated, and acetolyzed pollen gave similar results.

## DISCUSSION

Information obtained from scanning electron microscopy of *Zea* and *Iva* pollen has indicated direct correlations with that gathered from light microscopy of whole mounts and thin sections, and electron microscopy of thin sections. These results did not in any way put a new interpretation on past analyses, but did, however, help clarify, put into a better perspective, and support these data.

Although most scanning studies have tended to emphasize the ornamentation of the pollen wall *per se,* we feel that the information presented here on germinal apertures is also of great value. Dimensions of colpi and pores can be observed easily and with greater accuracy than with light microscopy. The utilization of these parameters in taxonomic problems is obvious.

While the scanning electron microscope is designed primarily to give surface data, it is interesting to note that we have also been able to observe pollen wall stratification. It is likely that techniques will be developed which will provide more such data. That this is already nearing reality is indicated by scanning photographs of pollen which has been sectioned (Echlin, 1968).

The present study emphasizes the necessity for preparing pollen by different methods in order to gain maximum information. We have found that while fresh, untreated pollen is suitable for study, it is necessary to use fixation ($OsO_4$) and acetolysis for supplementation. Use of $OsO_4$ to stabilize labile algal and microbial specimens has provided satisfactory results (Echlin, 1968). Since procedures of $OsO_4$ fixation are standard in pollen studies using the transmission electron microscope, by using $OsO_4$ preparations in our scanning electron microscope studies, we can make direct correlative comparisons which are otherwise not possible.

## REFERENCES

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